

appears that the VH56p1 expressing B cells are selected, perhaps by an endogenous pathogen or self antigen, in the naive animal. It may be relevant that this same gene is over-represented in the human fetal repertoire.

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Summary

Upstream cis-acting sequences define the functionality of the individual switch regions, and are necessary for class switching. Our observation--that class
 10 switching within the HC1 transgene is largely confined to cells involved in secondary response, and does not occur randomly across the entire B cell population--suggests that the minimal sequences contained with the transgene are sufficient. Because the γ sequences included in this
 15 construct begin only 116 nucleotides upstream of the start site of the γ 1 sterile transcript, the switch regulatory region is compact.

Our results demonstrate that these important cis-acting regulatory elements are either closely linked to
 20 individual γ genes, or associated with the 3' heavy chain enhancer included in the HC1 and HC2 transgenes. Because the HC1 and HC2 inserts undergo transgene-autonomous class switching--which can serve as a marker for sequences that are likely to have been somatically mutated--we were able to
 25 easily find hypermutated transcripts that did not originate from translocations to the endogenous locus. We found somatically mutated γ transcripts in three independent transgenic lines (two HC1 lines and one HC2 line). It is therefore unlikely that sequences flanking the integration
 30 sites of the transgene affect this process; instead, the transgene sequences are sufficient to direct somatic mutation.

EXAMPLE 36

This example describes the generation of hybridomas
 35 from mice homozygous for an inactivated endogenous immunoglobulin locus and containing transgene sequences encoding a human sequence heavy chain and human sequence light chain. The hybridomas described secrete monoclonal antibodies

comprising a human sequence heavy chain and a human sequence light chain and bind to a predetermined antigen expressed on T lymphocytes. The example also demonstrates the capacity of the mice to make a human sequence antibody in response to a human-derived immunogen, human CD4, and the suitability of such mice as a source for making hybridomas secreting human sequence monoclonal antibodies reactive with human antigens.

A. Generation of Human Ig Monoclonal Antibodies Derived from

10 HC1 Transgenic Mice Immunized with a Human CD4 Antigen

A transgenic mouse homozygous for a functionally disrupted J_H locus and harboring a transgene capable of rearranging to encode a human sequence heavy chain and a transgene capable of rearranging to encode a human sequence light chain was immunized. The genotype of the mouse was HC1-26⁺ KC1e-1536⁺ $J_H D^{+/+}$ $J_K D^-$, indicating homozygosity for murine heavy chain inactivation and the presence of germline copies of the HC1 human sequence heavy chain transgene and the KC1e human sequence light chain transgene.

20 The mouse was immunized with a variant of the EL4 cell line (ATCC) expressing a mouse-human hybrid CD4 molecule encoded by a stably transfected polynucleotide. The expressed CD4 molecule comprises a substantially human-like CD4 sequence. Approximately 5×10^6 cells in 100 μ l of PBS accompanied by 100 μ l of Complete Freund's Adjuvant (CFA) were introduced into the mouse via intraperitoneal injection on Day 0. The inoculation was repeated on Days 7, 14, 21, 28, 60, and 77, with test bleeds on Days 18, 35, and 67. The spleen was removed on Day 81 and approximately 7.2×10^7 spleen cells were fused to approximately 1.2×10^7 fusion partner cells (P3x63Ag8.653 cell line; ATCC) by standard methods (PEG fusion) and cultured in RPMI 1640 15 % FCS, 4 mM glutamine, 1 mM sodium pyruvate plus HAT and PSN medium. Multiple fusions were performed.

35 Hybridomas were grown up and supernatants were tested with ELISA for binding to a commercial source of purified recombinant soluble human sequence CD4 expressed in CHO cells (American Bio-Technologies, Inc. (ABT), Cambridge,